

# A Role for Associated Transition Metals in the Immunotoxicity of Inhaled Ambient Particulate Matter

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Epidemiologic studies demonstrate that infection, specifically pneumonia, contributes substantially to the increased morbidity and mortality among elderly individuals following exposure to ambient particulate matter (PM). This laboratory has previously demonstrated that a single inhalation exposure of *Streptococcus pneumoniae*-infected rats to concentrated ambient PM<sub>2.5</sub> (particulate matter with aerodynamic diameter  $\leq 2.5 \mu\text{m}$ ) from New York City (NYC) air exacerbates the infection process and alters pulmonary and systemic immunity. Although these results provide some basis for explaining the epidemiologic findings, the identity of specific PM constituents that might have been responsible for the worsening pneumonia in exposed hosts remains unclear. Thus, studies were performed to correlate the physicochemical attributes of ambient PM<sub>2.5</sub> with its *in vivo* immunotoxicity to identify and characterize the role of constitutive transition metals in exacerbating an ongoing streptococcal infection. Uninfected or previously infected rats were exposed in the laboratory to soluble divalent Fe, Mn, or Ni chloride salts. After exposure, uninfected rats were sacrificed and their lungs were lavaged. Lungs from infected hosts were used to evaluate changes in bacterial clearance and effects of exposure on the extent/severity of infection. Results demonstrated that inhalation of Fe altered innate and adaptive immunity in uninfected hosts, and both Fe and Ni reduced pulmonary bacterial clearance in previously infected rats. The effects on clearance produced in infected Fe-exposed rats were similar to those seen in infected rats exposed to ambient NYC PM. Taken together, these studies demonstrate that inhaled ambient PM can worsen the outcome of an ongoing pulmonary infection and that associated Fe may play some role in the immunotoxicity. **Key words:** air pollution, immunotoxicity, inhalation, metals, particulate matter, pulmonary immune defenses. *Environ Health Perspect* 110(suppl 5):871–875 (2002).

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Epidemiologic studies have reported that exposure to ambient levels of airborne particulate matter with an aerodynamic diameter of  $\leq 10 \mu\text{m}$  (PM<sub>10</sub>) results in consistent increases in morbidity and mortality (1–3), and that elderly individuals seem to be particularly affected (4,5). Infection, specifically pneumonia, contributes substantially to the mortality among elderly individuals exposed to PM, and disproportionate increases in deaths due to pneumonia have been observed immediately or just after even moderate episodes of particulate air pollution (6,7). These epidemiologic findings suggest that PM may act as an immunosuppressive factor that can undermine the normal pulmonary immune response. Thus, given that older individuals with chronic respiratory disease are not only at increased risk of pneumonia but also are less likely to recover from infections (8), alterations in the pulmonary immune system may well play a role in the observed increase in mortality following PM episodes.

Toxicologic studies demonstrating the immunosuppressive potential of particles in the lungs strengthen the epidemiologic observations and support the hypothesis that compromised pulmonary host immunocompetence and immune defense mechanisms important for resistance against *Streptococcus pneumoniae* infections contribute to the

observed increase in particle-induced mortality in elderly individuals. Studies using rodent models have clearly demonstrated that exposure to inhaled particles (alone or in combination with gaseous air pollutants) can compromise pulmonary host resistance against microbial infections and/or alter specific immune mechanisms important for antibacterial defense. For example, Aranyi et al. (9) demonstrated that intratracheal (IT) instillation of mice with either quartz, ferric oxide, calcium carbonate, or sodium feldspar particles increased mortality from subsequent infection with *S. pneumoniae*. In other bacterial infectivity studies, instillation of aged urban air particles (0.4  $\mu\text{m}$ , mass median aerodynamic diameter [MMAD]) and/or coal fly ash particles (0.9  $\mu\text{m}$  MMAD) reduced the resistance of mice to bacterial infection (10). Moreover, studies in this laboratory demonstrated that inhalation of woodsmoke effluents reduced pulmonary clearance of IT-instilled *Staphylococcus aureus* (11).

Although it has been demonstrated that the elderly with preexisting disease appear to be at higher risk from the adverse effects of PM than healthy individuals, considerable uncertainty remains about specific biologic mechanisms that might underlie this effect. Thus, studies were performed in which rats

previously infected with *S. pneumoniae* were subsequently exposed to concentrated PM with aerodynamic diameter  $\leq 2.5 \mu\text{m}$  (PM<sub>2.5</sub>) (at a level at or just above the PM<sub>2.5</sub> National Ambient Air Quality Standard [NAAQS]) from New York City (NYC) to determine whether acute exposure to PM induces immunologic alterations within the lungs that could exacerbate an ongoing *S. pneumoniae* infection. Results from these studies demonstrated that a single inhalation exposure of ambient PM<sub>2.5</sub> worsened disease outcome and compromised both local and systemic immune defense mechanisms in exposed animals (12).

Studies were also performed to determine which constituent(s) might be responsible for the observed worsening of pneumonia in PM-exposed hosts. Based upon results from a number of *in vivo* and *in vitro* investigations demonstrating the role of particle-associated transition metals in mediating PM-related health effects (13–16), as well as the ability of some of the same metals to suppress host immunocompetence (17), experimental studies were performed to characterize the role of constitutive transition metals in exacerbating ongoing pneumococcal infections.

## Materials and Methods

### Experimental Animals

Pathogen-free male Fischer 344 rats (Harlan Sprague Dawley, Indianapolis, IN, USA) 7–9 months of age were quarantined for at least 1 week prior to use in any experiments. Rats were housed individually in stainless steel cages in temperature- and humidity-controlled rooms and provided food and water *ad libitum*. In addition to serology testing for viral and bacterial pathogens, rats were examined routinely during the exposure studies for any gross indication of spontaneous infection or for mucosal irritation due to exposure.

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Infected rats were maintained individually in HEPA filter-top cages and housed in an onsite Biosafety Level 2 facility. Animals used in this research have been treated humanely according to institutional guidelines.

### Experimental Design

Male rats previously infected by IT instillation with  $15\text{--}20 \times 10^6$  *S. pneumoniae* were exposed for 5 hr to either ambient NYC PM<sub>2.5</sub> (65–90  $\mu\text{g}/\text{m}^3$ ) or to a single PM-associated transition metal (i.e., iron [Fe<sup>2+</sup>], manganese [Mn<sup>2+</sup>], or nickel [Ni<sup>2+</sup>]) of a similar concentration and MMAD (i.e., 0.4  $\mu\text{m}$ ;  $\Sigma g = 2.4 \mu\text{m}$ ) and then sacrificed at different time points postexposure. Effects of inhaled transition metals were also determined in uninfected rats. At the time of sacrifice, lungs were *a*) lavaged to provide fluid for evaluation of markers of lung cell damage (i.e., lactate dehydrogenase [LDH] and total protein) or to provide cells for characterization; *b*) fixed for histopathologic examination; or *c*) homogenized for determination of effects on pulmonary bacterial burdens. Blood taken from the portal vein prior to sacrifice was used to determine relative percentages of circulating white blood cells. A total of 100 white blood cells per slide (two slides per preparation) were counted for differential counts.

### Exposures

Ambient PM and filtered air exposures took place in Teflon nose-only exposure units (CH International, Westwood, NJ, USA) located in a building overlooking a main thoroughfare in Manhattan, NYC. Concentrated ambient PM<sub>2.5</sub> air was produced using a Gerber centrifugal concentrator; gaseous pollutants such as ozone, sulfur dioxide, nitrogen dioxide, and ammonia were removed prior to exposure (18). The exposure atmosphere was continuously monitored using a condensation particle counter and real-time aerosol monitor. In addition, filter samples were collected during exposure on Teflon membrane filters to gravimetrically measure integrated exposure mass concentrations on a Cahn electrobalance.

For the individual metal exposure studies, previously infected and naive rats were exposed by inhalation (i.e., nose-only) for 5 hr to chloride salts of either Fe, Mn, or Ni at a concentration of 65–90  $\mu\text{g}/\text{m}^3$ . All atmospheres were generated by passing freshly prepared solutions of each metal compound through a collision nebulizer; generated particles were then delivered to each rat held in individual body tubes (CH International) on a single-exposure tree (12).

### Pulmonary Bacterial Clearance

Six days prior to instillation, *S. pneumoniae* (encapsulated, type 3) was introduced

into fresh Todd Hewitt (TH) broth and maintained at 37°C in a 5% CO<sub>2</sub> atmosphere. The cultures were then passaged at 12-hr intervals using a protocol that maintained both the virulence and capsulated nature of the organism. On the day of instillation, the bacterial concentration was spectrophotometrically determined using an absorbance calibration curve prepared at 540 nm and a suspension diluted with phosphate-buffered saline (PBS) to a suitable concentration for delivery of  $1\text{--}2 \times 10^7$  organisms in a 100- $\mu\text{L}$  volume.

At designated time intervals, cohorts of four rats each were sacrificed by injection of Nembutal (sodium phenobarbital; 80 mg/kg, sc) and the lungs were removed, weighed, and homogenized (19). To obtain estimates of total remaining viable organisms, aliquots of the homogenate were serially diluted and plated onto triplicate sheep blood-TH agar plates for a 24-hr incubation at 37°C (in 5% CO<sub>2</sub>) before enumeration. Both the absolute levels of bacteria and the levels of bacteria per gram lung compared with those in three randomly infected rats sacrificed immediately prior to beginning inhalation exposure were used as indices of bacterial survival.

### Bronchopulmonary Lavage and Biologic Assays

At sacrifice, lungs from uninfected rats were lavaged by washing the left lung *in situ* twice with Ca<sup>2+</sup>- and Mg<sup>2+</sup>-free PBS according to previously employed methods (20). Spleens were also recovered and placed in RPMI 1640 on ice until processed for measurement of lymphoproliferative responses. Aliquots of acellular lavage fluid were then used to evaluate LDH activity and total protein (21). Lavaged cell numbers and viability were determined by hemocytometer counting and trypan blue exclusion, respectively. Recovered lavaged cell types were subsequently characterized morphologically by differential counting of stained cells. Basal and serum-opsonized, zymosan-stimulated production of superoxide anion (O<sub>2</sub><sup>•-</sup>) by pulmonary macrophages (M $\phi$ ) was assessed using a microtiter plate assay based upon the reduction of ferricytochrome *c* (20). Proliferation of splenic T and B lymphocytes was measured in response to stimulation with concanavalin (Con) A and lipopolysaccharide (LPS), respectively (22).

### Statistical Analyses

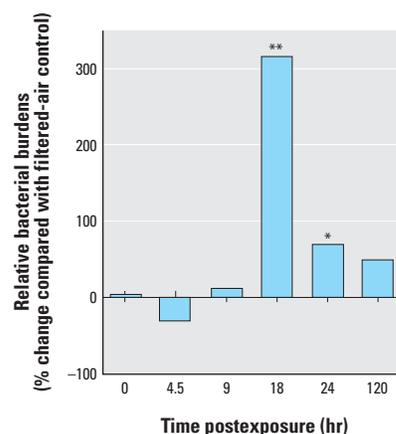
The effects of PM exposure itself upon each of the assayed parameters (i.e., exposed vs. air control), as well as those effects associated with length of time post-PM exposure, were analyzed using a two-way analysis of variance. For outcomes such as bacterial clearance,

which were measured on a percentage scale, the need for arcsine transformation was determined prior to analysis. Differences were considered significant at  $p < 0.05$ .

## Results

In studies examining the effects of concentrated ambient PM (CAPS), previously infected rats were sacrificed 4.5, 9, 18, 24, and 120 hr after exposure, and effects upon bacterial burdens were determined (Figure 1). Results demonstrated that although numbers of pulmonary bacteria were approximately equal in the two exposure groups at the earliest postexposure time point (i.e., 4.5 hr postexposure), bacterial burdens in the CAPS-exposed animals were approximately 10% above those measured in the air controls by 9 hr; by 18 hr, burdens were elevated >300%. After 24 hr CAPS-exposed rats had substantially greater (i.e., 70% change from control) bacterial burdens than infected control rats. At 5 days postexposure, total number of bacteria per gram lung was still 30% above that measured in the lungs of the infected air-exposed controls.

Uninfected rats exposed nose-only to either Fe, Mn, or Ni at 65–90  $\mu\text{g}/\text{m}^3$  demonstrated significant alterations in blood cell profiles (Figure 2A, B, C, respectively). Although polymorphonuclear leukocyte (PMN) levels significantly increased,



**Figure 1.** A single exposure of already-infected rats to CAPS significantly increased pulmonary bacterial burdens of *S. pneumoniae* in a time-dependent manner (compared with the infected, air-exposed control). Estimates of viable bacterial organisms were determined from colony counts on blood-TH agar 24 hr after plating. Both the absolute levels of bacteria and the levels of bacteria per gram lung compared with those in three randomly-infected rats sacrificed immediately prior to beginning inhalation exposure were used as indices of bacterial survival. Postexposure time 0 represents those burdens determined immediately prior to exposure. Asterisk (\*) indicates value significantly different from filtered-air control ( $p < 0.05$ ). Double asterisks (\*\*) indicate significantly different from filtered-air control ( $p < 0.01$ ).

lymphocyte values significantly decreased at 1 hr postexposure; however, by 18 hr postexposure, leukocyte values reached control levels. Acute inhalation of Mn and Fe had no effect upon lavageable cell number or lung histologic profile, and none of the metals altered cell viability or LDH activity (compared with control). In contrast, inhalation of Ni significantly reduced lavaged cell numbers by 25% (i.e.,  $40 \times 10^6$  vs  $30 \times 10^6$  cells for control and Ni-exposed rats, respectively) and increased percent lung involvement and alveolar edema/exudate 1 hr postexposure (data not shown).

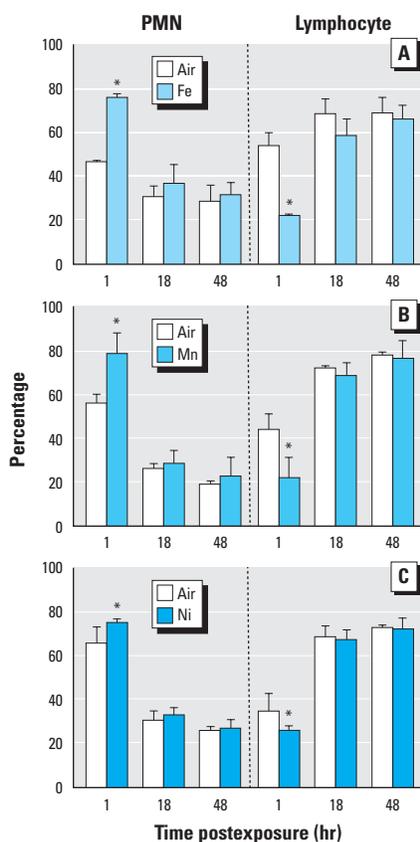
Metal exposure also altered certain pulmonary and systemic immune functional activities in uninfected animals. Inhalation exposure to Fe significantly increased (compared with time-matched air controls) basal production of  $O_2^{\cdot-}$  by lavaged M $\phi$  18 and 48 hr postexposure (Figure 3); inhalation of Fe had no effects on  $O_2^{\cdot-}$  production by stimulated M $\phi$  at any postexposure time point. Although exposure of naive rats to Mn had no effect upon lymphoproliferation (data not shown), inhalation of Fe or Ni significantly altered the ability of splenic lymphocytes to proliferate in response to mitogen stimulation (Figure 4A, B). Inhalation of Fe significantly reduced B-lymphocyte proliferation in response to LPS stimulation 48 hr postexposure, but had no effect upon Con A-stimulated T-lymphocyte proliferation at any postexposure time point (Figure 4A); at 48 hr postexposure, inhaled Fe suppressed unstimulated T-lymphocyte proliferation. On the other hand, T cells proved more sensitive to the immunotoxic effects of inhaled Ni than did B lymphocytes. T-cell proliferation was significantly reduced (compared with the air control) by inhaled Ni 18 hr postexposure and returned to control levels after 48 hr; B-cell responses to LPS stimulation were uniformly unaffected by Ni exposure (Figure 4B).

Figure 5 illustrates the effects of inhaled Mn, Ni, and Fe on pulmonary bacterial clearance. Although a single inhalation of Mn had no significant effect upon bacterial lung burdens (as represented as total burdens) compared with the time-matched air controls (Figure 5A), exposure to Ni or Fe significantly altered bacterial clearance (Figure 5B, C, respectively). Although pulmonary pneumococcal levels in air-exposed infected rats dropped significantly 18 hr following exposure (compared with burdens measured in rats sacrificed immediately prior to exposure), exposure to Ni inhibited clearance and bacterial burdens remained the same as those measured just prior to metal exposure (Figure 5B). Conversely, inhalation of Fe increased pulmonary streptococcal levels compared with preexposure bacterial burdens (Figure 5C). Thus, whereas exposure to Ni appeared

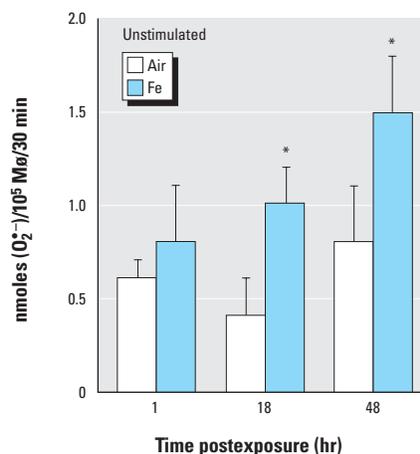
to inhibit clearance, inhalation of Fe facilitated an increase in overall bacterial numbers.

Inhalation of Fe by infected rats also altered lavageable cell numbers and immune cell profile compared with those of infected

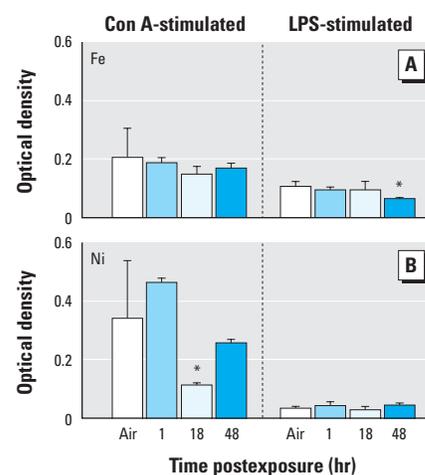
air-exposed control animals (Table 1). Although lung cell viability was unaffected at 18 hr postexposure, lavageable cell numbers in the Fe-exposed infected rats decreased by 35% compared with levels in time-matched



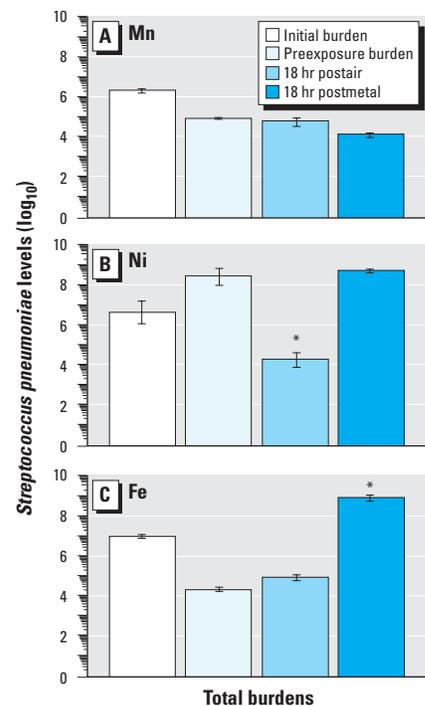
**Figure 2.** Inhalation (i.e., nose-only) of Fe (A), Mn (B), or Ni (C) by uninfected rats alters circulating blood cell profiles. Each bar represents the mean ( $\pm$  SD) from four rats per exposure group. Asterisk (\*) indicates value significantly different from time-matched air control ( $p < 0.05$ ).



**Figure 3.** Inhalation of Fe by uninfected rats increased unstimulated  $O_2^{\cdot-}$  production by lavaged M $\phi$  18 and 48 hr postexposure. Each bar represents the mean ( $\pm$  SD) from four rats per exposure group. Asterisk (\*) indicates value significantly different from time-matched air control ( $p < 0.05$ ).



**Figure 4.** Inhalation of Fe (A) and Ni (B) by naive rats alters proliferative responses of mitogen-stimulated B and T lymphocytes, respectively. Each bar (except pooled air control, which represents the mean of 12 rats) represents the mean ( $\pm$  SD) from four rats per exposure group. Asterisk (\*) indicates value significantly different from individual time-matched air control ( $p < 0.05$ ).



**Figure 5.** Burdens of *S. pneumoniae* in the lungs of Mn- (A), Ni- (B), and Fe- (C) exposed infected rats 18 hr postexposure. Estimates of viable bacterial organisms were determined from colony counts on blood-TH agar 24 hr after plating. Absolute levels of bacteria were used as indices of bacterial survival. Each bar represents the mean ( $\pm$  SD) from five rats per exposure group. Asterisk (\*) indicates value significantly different from time-matched air control ( $p < 0.05$ ).

**Table 1.** Inhalation of Fe by *Streptococcus pneumoniae*-infected hosts alters lavageable lung immune cell numbers and profiles.

Treatment	Hours post-infection <sup>a</sup>	Hours post-exposure	Total cells recovered (× 10 <sup>7</sup> )	Viability (%)	Cell type (%) <sup>b</sup>		
					PMN	Lymphocyte	Macrophage
Air	51	3	3.3 ± 1.0	86.0 ± 4.0	30.0 ± 14.0	9.0 ± 0.2	60.0 ± 14.0
Fe	51	3	2.6 ± 1.0	84.0 ± 3.0	25.0 ± 2.0	14.0 ± 6.0	61.0 ± 4.0
Air	66	18	2.6 ± 0.1	87.0 ± 2.0	26.0 ± 9.0	11.0 ± 2.0	63.0 ± 7.0
Fe	66	18	*1.7 ± 0.3	83.0 ± 5.0	*8.0 ± 7.0	*4.0 ± 2.0	*88.0 ± 6.0

<sup>a</sup>Rats were infected with 10–20 × 10<sup>6</sup> *S. pneumoniae*. <sup>b</sup>Mean ± SD of five rats per exposure group. \*Indicates significant difference ( $p < 0.05$ ) from time-matched air control.

air controls. At this same time point, relative percentages of lavageable PMNs and lymphocytes in Fe-exposed rats dropped approximately 3-fold, whereas Mø values increased by 29%.

## Discussion

Studies to determine whether inhalation exposure to concentrated PM<sub>2.5</sub> could exacerbate an ongoing pneumococcal infection demonstrated that a single 5-hr exposure of *S. pneumoniae*-infected rats to CAPS (at concentrations at or slightly greater than 65 µg/m<sup>3</sup>) exacerbated the infection process in a time-dependent manner and altered both pulmonary and systemic immunity (12). This was not surprising, given that lungs containing extant pulmonary inflammation appear to be primed for injurious responses to air particles (23). It appears from these studies that CAPS may be acting to alter lung antibacterial defense mechanisms important in the handling of ongoing pneumococcal infections. This scenario fits temporally with the epidemiologic data that indicate that deaths among exposed individuals occur relatively quickly following a PM episode, and that individuals with chronic respiratory disease were less likely than healthy individuals to recover from pulmonary infections following PM exposure (1,24, 25).

Although no definitive conclusions can be reached at this time regarding the mechanism(s) by which inhaled concentrated PM<sub>2.5</sub> may have acted to increase pulmonary burdens of infectious pneumococci, compromised pulmonary immune defense mechanisms important for the removal/killing of *S. pneumoniae* (i.e., modified availability of PMNs) or increased bacterial survival as a result of CAPS-induced changes in the lung milieu are two plausible hypotheses by which this may have occurred. In support of the former, CAPS exposure of rats with ongoing pneumonia reduced the relative percentages of lavageable PMNs 24, 48, and 72 hr postexposure compared with that observed in the air-exposed infected controls (12). Given that PMNs, in the presence of opsonizing complement and immunoglobulins, are the main cell type responsible for the clearance of *S. pneumoniae* from the lungs of most animal models and humans (26), a PM-induced reduction could lead to prolongation of infection.

Decreased percentages of PMNs may have been due, at least in part, to the previously observed CAPS-induced downregulation of tumor necrosis factor (TNF)α and/or interleukin (IL)-1α production (12); both of these cytokines are critical for the mobilization and activation of PMNs in response to many Gram-positive [G<sup>+</sup>] microbial organisms including *S. pneumoniae* (27). Moreover, since TNFα in conjunction with IL-12 enhances the microbicidal capacity of PMNs (28), the immune cells still present in the lungs may display reduced cytotoxic activities.

Inorganic constituents of airborne PM such as sulfate, nitrate, ammonium, and transition metals, which make up a substantial part of the mass apportionment of ambient PM, represent potential causal constituents for PM-associated health effects. Although a number of different physicochemical factors have been linked to PM toxicity (i.e., acid aerosols, particle size, oxidative potential), evidence is rapidly accumulating that much of the pulmonary toxicity associated with inhaled PM is related to the types and amounts of the soluble forms of transition metals (13,14,16).

Metals are ubiquitous constituents of PM derived from anthropogenic and certain types of natural emissions. PM emissions from oil-burning power plants and other industries that contribute to air pollution contain large amounts of metals such as V, Fe, Ni, Zn, and Cu (15,16). Moreover, studies from this laboratory have demonstrated the presence of Mn in concentrated NYC PM (29). Although inflammation was not observed in this study, human exposure to airborne metals have been shown to induce pulmonary inflammatory responses such as tracheobronchitis, asthma, chemical pneumonitis, and alveolitis. Recent studies have shown that acute exposure of rats to mixtures of metallic compounds derived from ambient air PM or from combustion source emissions (i.e., residual oil fly ash) can induce pronounced pulmonary inflammation characterized by increased permeability to protein and neutrophilic alveolitis (13).

Many transition metals associated with PM are potent modulators of pulmonary and/or systemic immunocompetence (30). For example, both parenteral and inhalation exposure of soluble NiCl<sub>2</sub> causes activation

of alveolar Mø followed within 2 days by suppressed phagocytic activity and enhanced lipid peroxidation (31). Similar findings have also been observed in rabbits exposed by inhalation of Ni dust for 1–6 months (32). Alveolar Mø recovered from rabbits exposed to NiCl<sub>2</sub> aerosol for 1 month also displayed suppressed phagocytic ability as well as decreased levels of lysozyme (used by Mø to break down G<sup>+</sup> bacterial cell walls) both in pulmonary phagocytes and bronchopulmonary lavage fluid. Moreover, a number of studies have concluded that inhalation/instillation of certain Ni compounds, including NiCl<sub>2</sub>, reduces host ability to defend against pathogenic lung infections (30). Given that lysozyme activity has been correlated with the ability to clear certain G<sup>+</sup> bacterial lung pathogens, and that the major cellular target of inhaled Ni appears to be Mø (and other antigen presenting cells), it is likely that Ni-induced effects upon antibacterial defense may be due, at least in part, to suppressive effects on this phagocyte-associated enzyme.

Fe is a key microelement necessary to maintain cellular homeostasis. Normal functioning of the immune system relies on trace amounts of Fe to serve as a cofactor for specific metalloenzymes and for the intracellular formation of reactive oxygen species used for killing phagocytosed pathogens. Both deficiency and excessive levels of Fe can lead to immune dysfunction (33). Although most studies have focused upon the immunomodulating effects associated with deficiency, Fe overload has been shown to suppress antibody responses, T-lymphocyte functions (i.e., interferon-γ production and delayed contact hypersensitivity), and nonspecific immunity (33). For example, bactericidal activities of Mø from patients with Fe overload and of leukocytes treated *in vitro* with Fe salts were markedly reduced (34); inhibition of basic cationic proteins appeared to be responsible for these reductions. In contrast, results from experimental studies have suggested that Fe-overloaded Mø have improved bactericidal ability (35). Discrepancies between the studies may be due to differences in host species and/or the particular microorganism being targeted by the phagocyte. More consistent effects of Fe excess have been observed on natural killer cell cytotoxicity and neutrophilic

killing of *S. pneumoniae*. As has been observed in clinical and experimentally induced Fe deficiency (36), patients with clinical diseases of Fe overload (i.e., thalassemia major) have decreased bactericidal, fungicidal, and oxidative burst activities (33); neutrophil-associated oxidative burst activity was also diminished in proportion to the degree of Fe overload. Although inhaled Fe may have led to increased pulmonary burdens of *S. pneumoniae* in this study by interfering with phagocyte-mediated killing, another possibility is that freely available inhaled Fe may have eventually overwhelmed the binding capacities of the nascent Fe-binding proteins transferrin and lactoferrin normally operational in lung fluid (37), thereby allowing bacteria that require Fe as an essential nutrient (including *S. pneumoniae*) to freely incorporate the metal and proliferate.

Mn, a dissociable cofactor for several enzymes including one of great importance in the lungs, superoxide dismutase, is also required for growth of virtually all living cells. Like Fe, shifts in Mn levels in either direction can bring about immune dysfunction. Inhalation of high concentrations of Mn by occupationally exposed workers has been shown to cause Mn pneumonitis and croupous pneumonia. In rodents, inhalation of insoluble Mn at milligram concentrations impairs pulmonary bacterial clearance and increases bacterial-associated host mortality (30). Other studies have demonstrated that exposure of hosts already bearing a viral lung infection for 24 or 48 hr prior to a 3-hr exposure to insoluble Mn had a shorter time to death than their air-exposed counterparts (38,39). Furthermore, although the effects of soluble Mn on alveolar MØ function are still being debated, *in vitro* studies have demonstrated reductions in phagocytic activity. Discrepancies between these studies and those performed herein that demonstrated no effects of inhaled Mn on pulmonary bacterial clearance may be due, in part, to differences in metal concentration and/or metal solubility.

Taken together, findings from this study support the notion that a single exposure to concentrated ambient PM<sub>2.5</sub>, at concentrations equal to or just above the promulgated 24-hr NAAQS value, compromise host ability to adequately handle an ongoing *S. pneumoniae* infection. These investigations also provide biologic plausibility for the role of PM-associated metals, particularly Fe and Ni, in exacerbating *S. pneumoniae* infections in CAPS-exposed hosts. Although more research

is needed to conclusively confirm or refute the role of transition metals in PM-associated immunosuppression, results contribute to a better understanding of the possible mechanism(s) by which exposure to PM<sub>2.5</sub> may act to increase host mortality in exposed elderly individuals.

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